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Induced Division of Leucocytes and the Genesis of Tumours.[1]

By Sir RONALD ROSS, K.C.B., F.R.S.

SCIENTIFIC workers may like to have a brief account of some recent researches which, I think, are likely to be of both theoretical and practical interest. The researches commenced nearly five years ago in a special study of leucocytes by a method devised by my brother, Mr. H. C. Ross, and myself. This consists in placing liquid blood under a coverglass, not as usual upon another surface of glass, but upon a bed of transparent jelly with which various reagents, including stains, have been mixed. The original object of the method was to try to cultivate human leucocytes *in vitro*. The jelly prevents mechanical injury to the blood-cells by pressure, disposes them in a beautifully regular film, and at the same time causes them to be uniformly affected, when living, by the reagents—the whole process being continuously visible under the microscope. At first, careful studies of the rate of absorption of stains by the leucocytes under various chemical conditions of the jelly were made by Mr. Ross; but during this investigation he discovered that atropine and some other alkaloids cause these cells to emit and retract long processes in a curious manner. Two years later he found that extract of hæmal gland, extracts of apparently many dead and decomposing tissues, and globin, when mixed with the jelly, force a large proportion of the leucocytes to divide before the eyes. Subsequently he and his assistant, Dr. J. W. Cropper, ascertained by a series of lengthy studies that many of the substances which possess this property (in different degrees) belong to the amidine grouping. They have found also that a second series of substances, though by themselves they cannot produce division of leucocytes, have the power of augmenting very greatly the power

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of the former group of substances to do so. They give the names *auxetics* and *augmentors* to the two groups respectively. The principal auxetics are extracts of organs—creatine, xanthine, creatinine, guanidine, benzamidine, theobromine, acetamidine, caffeine, theophylline, methylamine, ethylamine, propylamine, &c., and certain aniline dyes. Some of the augmentors are various alkaloids—atropine, choline, cadaverine, neurine, &c.

The technique, though simple, requires considerable care, because exact amounts of salts, auxetics and augmentors must be mixed with the jellies, and the specimens must be kept at the proper temperature from the moment that the blood is drawn from the subject. If a stain, such as polychrome methylene blue, is added the cells become coloured progressively as the division advances. All the varieties of the human leucocytes can be made to divide, but the technique is slightly different for each variety. The proportion of cells affected in a given preparation of blood varies according to perfection of technique up to, say, 80 per cent.; but as death occurs rapidly, especially if stain be used, it usually overtakes a large proportion of them before the division has been completed. After about twenty minutes all the cells die; and by that time the process is complete in only a small percentage. Efforts to keep the cells alive longer upon these medicated jellies, or in solutions of auxetics, have not yet been very successful, and would not be easy. After their death the leucocytes give up again most of their stain, and the jelly preparation rapidly spoils; but a method has been found of making (with some difficulty) permanent specimens of such of the blood as adheres to the coverglass by fixing the whole preparation with osmic acid vapour, and then freezing and picking off the coverglass from the bed of jelly. In such specimens the cells retain the form which they happened to possess at the moment of fixation, and can then be stained when dry.

To watch the same cell passing through the whole process requires an accurately adjusted warm stage or microscope incubator and considerable patience, because the cell which we happen to select for observation will most probably belong to the majority which die before completion of the division; but partial division can be easily witnessed. If, however, the specimen is incubated for ten minutes, and is then surveyed rapidly from field to field, numbers of the leucocytes caught in all stages of the process can be readily seen. The fixed films just referred to show exactly the same objects, but enable us to examine them repeatedly and at leisure. And in both these cases the dividing

forms are so numerous and similar that there can be no question of their being exceptional artefacts or distortions, such as may sometimes simulate almost anything. Division of the mononuclear variety of leucocytes is produced and studied the most easily, but that of the other varieties can with patience be seen quite frequently enough to leave no doubt of the nature of the process.

In films in my possession numerous examples of dividing mononuclears fixed at all stages demonstrate (together with observations of the jelly preparations) the following steps in the process. In a few minutes after the blood is drawn from the subject and mounted, the round so-called nucleus becomes oval and then kidney- or bean-shaped, leading on rapidly to the outline of two circles cutting each other, and lastly touching each other in a "figure of eight." When the process is about half complete, and if the direction of division is parallel to the surface of the jelly or glass, another phenomenon is seen. About four to eight finger-like processes, radiating from the point midway between the centres of the two circles, are protruded or divided off, giving the whole body roughly the appearance of an ant, of which the head and abdomen are simulated by the two spheres, and the legs by the processes just mentioned. Such forms are numerous and characteristic; but, of course, when the direction of division happens to lie at an angle to the surface they are foreshortened, or may be distorted by the pressure of the jelly. As the division proceeds the processes are retracted into each daughter sphere, until the final figure of eight is produced.

If polychrome methylene blue is put in the jelly the cells become coloured progressively as the division advances. At first, after a few minutes, the so-called cytoplasmic, or Altmann's, granules take a purple tinge; then the so-called nucleus becomes a pale blue; and last of all the so-called nucleolus is stained—after which, apparently, the cell dies. As the so-called nucleus proceeds to take the hour-glass and figure of eight forms, strands of coloured substance are seen, especially in the fixed films, passing between the two daughter spheres; and such connexions are maintained until complete dissociation occurs. Though in the last case it is often, of course, difficult to say after the event whether we are not merely seeing two cells which have fallen accidentally into juxtaposition, yet the previous steps of the process are quite unmistakeable. The behaviour of the so-called nucleolus is not easy to follow because, as just noted, it does not stain until the cell dies and further division is checked. On the other hand, the behaviour of the so-called cytoplasmic granules must be described as very curious. In the middle

of the division they are found to number about eight (when they can be easily counted) and to lie, each one, at the end of one of the finger-like processes mentioned above; and appearances suggest that half of each granule is distributed to one daughter cell, and the other half to the other daughter cell. No distinct chromosomes are seen at any stage *inside* the so-called nucleus; and there appears to be no sign of astral fibres—though perhaps the finger-like processes may be interpreted as being bunches of these fibres which have not been rendered individually visible by the process of staining employed. On conclusion of the process the so-called cytoplasmic granules appear to be equally distributed between the two daughter cells, and to place themselves on the outer surface of the so-called nucleus of each—that is, in the position in which they were seen in the original parent cell. Apparently asymmetric forms are also frequently seen, but need not be described here.

Such seem to me to be the facts as observed by myself in preparations shown or given to me by Mr. Ross and Dr. Cropper. I will not touch here upon the similar divisions of the so-called polymorphonuclear leucocytes—which have also been already described and figured by Mr. Ross. Nor will I attempt to reconcile the observations with current cytological teaching, even as regards the division of leucocytes. Very probably, different methods of staining may bring them, at least partly, into closer conformity. Though engaged for years in the study of blood I have never seen these forms before; nor, indeed, have I ever seen in any ordinary preparation what could certainly be called a dividing leucocyte. I have been shown bodies claimed to be such, but these are admittedly so rare that they are open to the usual logical fallacies connected with very exceptional observations. The observations here referred to are not open to these fallacies. As I have said, the dividing forms are so numerous and characteristic that we can have no doubt that they really are dividing forms—whatever other observations or theories may be on record. It seems to me, therefore, that we are now compelled to admit two new facts: (1) that large numbers of human leucocytes can be made to divide *in vitro;* and (2) that this division occurs entirely, or at least specially, in the presence of certain chemical substances.

Such were, in brief, the laboratory experiments; but Mr. Ross suggests that they may already be employed for some tentative generalizations which may be useful as working hypotheses. Subject to correction, I think it was usually held till some years ago that cell proliferation is principally a function of the cell itself, essentially

independent of its environment, though often stimulated by temperature, abundance of food, and so on—that is, that most cells divide when mature because it is their nature to do so. In 1900, however, J. Loeb showed that parthogenesis can be induced in the eggs of sea-urchins (Arbacia) by the addition of a definite proportion of $MgCl_2$ to sea-water; and since then many workers have studied such phenomena among other animals, while Wassilieff has used hyoscyamine, nicotine, and strychnine for similar researches. The independent observations now recorded would appear to extend cognate principles to body-cells, by showing that the division of leucocytes may be suddenly forced on at a great rate and in a few minutes by the absorption or presence of appropriate chemical agents, and may, perhaps, be inhibited by other chemical agents. Why are the leucocytes never found dividing in normal peripheral blood? Probably (as suggested by Loeb in regard to sea-water) because the auxetics are absent, or because there is some inhibitory substance in the serum (as indicated by some experiments). But why do they proliferate so rapidly at the site of an injury? The auxetics and augmentors are chemical substances, many of which are known to be formed in disintegrating or dead tissues; an injury is, therefore, likely or sure to produce them at the spot where it has occurred; and they will then compel the cells in the neighbourhood to divide as quickly as they compel similar cells to divide *in vitro*. And this hypothesis was supported at once by the simple experiment of placing auxetics upon small spots on the surface of ulcers. Wherever they were put profuse cell proliferation followed, as evident to the naked eye.

The further possible application of the hypothesis to the genesis of at least some kinds of tumours is as follows: It is very probable that many individuals contain in their tissues auxetics and augmentors derived from extensive cell death going on somewhere in the body, as, for instance, in consequence of chronic rheumatism, decaying teeth, old intestinal, mammary and uterine lesions, or even advancing age. In such persons any new, small internal or external injury might be followed by *overhealing* due to excess of auxetics in the general body plus those formed at the site of injury by the local cell death. Excessive cell proliferation would now occur; but the new cells being over-numerous would perish, would generate more auxetics, and would stimulate in their turn the production of still more new cells. Thus a vicious cycle would be established, and massive tumours such as we see in cancer might be gradually or rapidly built up. We should have

here, so to speak, the formation of a kind of internal scab—a process originally intended to be healing, but now perverted into being itself an excitant of its own malignant growth, and of metastatic growths elsewhere. There would be two factors, the general auxetic poisoning (so to speak) and the additional local auxetic poisoning; but it is conceivable that the second factor, if well established, might suffice to maintain the condition by automatically supplying the first factor—thus explaining the frequent success of transplantation of tumours. Conversely, excision may frequently cure by removing the local supply of auxetics. Very probably special kinds of cells require their own special kinds of auxetics and augmentors—possibly obtained from dead cells of their own kind; which may help to explain the numerous varieties of tumours met with.

It is impossible to consider in this brief note the pros and cons of the subject, or the relations which this theory bears to the numerous other excellent studies now being made on cancer, but the following point is of interest. It has been known for some time that warts and epithelioma are exceptionally common among men working with "gasworks tar-pitch," but, curiously enough, not among men working with "blast furnace tar-pitch." In fact, legislation is now proposed to deal with the former, but (expressly) not with the latter. Mr. Ross and Dr. Cropper have recently reported that, on extracts being made of these two substances and mixed with the jellies, the extract from the gasworks tar-pitch was found to be a powerful excitant of leucocyte division, while the extract of the blast furnace tar-pitch was not. Hypothetically, therefore, the constant application of the former to the skin would be likely to have just the effect which it is found to have. Of course, many more researches are required at various points of attack—and some of these are now being carried out under the McFadden Researches at the Lister Institute. The work will probably have many applications. Thus intestinal amœbæ have been shown to grow much more quickly in culture in the presence of some of these auxetics, and extract of dead leucocytes, when injected into men, has been shown to cause a considerable increase of the living cells.

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